Conventional and functional evaluation of semen in male dairy goats

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Abstract

Evaluating the reproductive indicators in sheep breeding in Colombia, particularly males, is essential to achieve optimal production parameters. This study aimed to evaluate the semen quality of male goats through both conventional and functional seminal analysis. Semen samples from eight goats were collected and conventional (volume, appearance, colour, motility, vigour, and morphology) and functional (plasma membrane integrity, mitochondrial membrane potential, DNA integrity, lipid peroxidation, and reactive oxygen species production) seminal parameters were evaluated. The results showed an average scrotal circumference of 25.69 cm, seminal volume of 0.51 mL, and concentration of 1936 x 10^6 sperm/mL. The average individual motility was 63.37%, vigour was four, and the percentage of abnormalities was 7.75%. Individual motility was correlated with the mitochondrial membrane potential (r=0.840, *P*=0.009) and reactive oxygen species production (r=-0.91, *P*=0.001). The average of cells with high mitochondrial membrane potential was 52.94%, while 39.29% were necrotic cells, the DNA fragmentation index average was 12.5%, reactive oxygen species production was 38.68%, lipoperoxidation analysis was 7.33% on average, and the integrity of the membrane was 54.77%. The results establish the parameters for the semen from Antioquia goats and confirm that goat semen is of good quality and could be used during artificial insemination programs.

Key words: *sperm; goat; reproduction; flow cytometry; semen*

Introduction

In genetic improvement programmes for goat populations, males are responsible for 60 to 80% of genetic progress since they have a more significant selection pressure, which determines that the study of the reproductive life

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of the male plays a fundamental role in breeding programmes. The reproductive examination of a male consists of a physical examination, and in some cases, the seminal quality is determined. In the first case, scrotal circumference, vision, poise, body condition, reproductive system, and visible anomalies are evaluated to detect altered bucks and thus diagnose possible infertility problems in the herd attributable to the male (Mesa Ortíz, 2012). In goat species, information regarding sperm quality is scarce compared to other species; previous studies reported the macro and microscopic parameters in the goat species: scrotal circumference (SC) varies between 24.97 to 28.11 cm (Morales et al., 2006; Revidatti et al., 2011), ejaculate volume 1.5 mL ± 0.46 mL, semen has a creamy appearance and white colour without the presence of any foreign material (Revidatti et al., 2011). The microscopic characteristics have been reported: total sperm concentration per ejaculate of 3000 to 6000 x 10^6 sperm/mL, normal morphology greater than or equal to 70%, and individual motility more significant than 60% (Nieto-Escorcia and Ruiz-Zarate, 2012). Additionally, mass motility and individual vigour are determined using a scale of 1 to 5, where 1 indicates immobile cells, and 5 is an intense wave movement in addition to the displacement force of the sperm (Nieto-Escorcia and Ruiz-Zarate, 2012).

The density of goat semen is due to the high sperm concentration. It presents an inverse relationship to the seminal volume, which, in turn, is correlated with the creamy appearance, white colour (the higher the concentration, the greater the colour intensity), and mass motility (the more significant the wave motion, the greater the sperm concentration) (Batista et al., 2006). The presence of high percentages of abnormal forms is associated with sexual immaturity, degenerative and pathological processes, and even with an excessive rate of semen collection, a good indicator of the spermatogenic capacity of a buck is its SC, which is directly related to sperm production (Balcázar and Porras, 2008).

On the other hand, the potential of new laboratory analysis methods has grown in recent years; among these, flow cytometry has been established to be a fast, simple, sufficiently sensitive, economic, and easily automated tool for growing academic needs, investigative and commercial. Flow cytometry is an automated, multiparametric, and quantitative method that allows information to be obtained on multiple cellular parameters (Otero, 2008). With flow cytometry, the quality, structure, and sperm function, damage to the sperm, and its quality for fertilisation can be evaluated (Laguado, 2007). Similarly, this technological development shows the importance of unifying efforts between the needs of the productive sector, private companies, and research centres to provide innovative solutions. For goat species, studies referring to flow cytometry are scarce; therefore, the implementation of flow cytometry is an innovative idea for sperm evaluation in male goats. Similar studies have been carried out in buffalo (Mayorga et al., 2013).

Therefore, the objective of this study was to evaluate the seminal quality of goats using a conventional and functional analysis to determine the physiological and reproductive status of the males, providing an innovative and more reliable alternative method for sperm evaluations.

Materials and methods

Ethical considerations

In compliance with the provisions of Resolution 008430 of 1993, the study was evaluated and endorsed by the

Ethics Committee for Experimentation with Animals (CEEA) of the University of Antioquia in Act No. 85/2014, which guarantees good practices in procedures carried out on goats under study and to guarantee their well-being throughout the process.

Type of study and population studied

A descriptive study was carried out on eight male goats (*C. aegagrus hirca*) from holdings in the Department of Antioquia (Table 1). This study was approved by the Ethics Committee for Experimentation with Animals (CEEA) of the University of Antioquia.

After obtaining the relevant permits from the owners, males were selected for dairy aptitude and riding rhythm. However, one male was selected for its libido, despite its meat aptitude.

Sample collection and evaluation

The physical examination of the locomotor system, scrotal circumference, body condition, vision, examination of the foreskin, penis, testicle (scrotum, epididymis, and spermatic cord), and ultrasound of the testicles were performed. Oestrogenised females were used as a lure to collect semen through the use of an artificial vagina, after which the macroscopic evaluation of the semen (volume, appearance, colour, and presence of abnormal content) was carried out, and the semen was kept at 35ºC until analysis.

Conventional seminal tests

The ejaculate was divided into two fractions: one for conventional analysis and the second for functional analysis. Conventional analysis consisted of using bright-field microscopy at 4x and 40x magnification to determine mass motility (on a scale from 1 to 5, where 1 indicates immobile cells and 5 are cells with a high percentage of mobility with intense wave movement). The semen was diluted in 0.9% saline solution (ratio 1:5), and tests of individual motility percentage and vigour were performed (scale 1 to 5, to evaluate the force of sperm displacement). The sperm concentration was quantified in millions/mL using the Neubauer chamber. Finally, morphology was assessed using an extended sample (10 µL semen) on a slide and stained with Giemsa, a total of 200 sperm were evaluated per slide according to sperm characteristics.

Table 1. Characteristics and location of the studied males

Holding	male	Breed	Location	Environment	
				T	E
A	1	Anglonubian	Medellín	22	1500
	2	Saanen x Alpine			
	3	Boer			
B	4	Alpine	El Retiro	16	2175
	5	Lamancha			
C	6	Alpine	La ceja	18	2143
D	7	Saanen	Caldas	20	1800
	8	Saanen			

T: temperature (in °C); E: elevation (in metres)

Functional seminal tests

The second fraction of the ejaculate was mixed 1:5 with the commercial diluent Triladyl (Minitube, Ontario, Canada) with 2% egg yolk. Functional tests using flow cytometry (Coulter Epics XL - Beckman Coulter, CA, USA) analysing between 5,000 and 10,000 sperm.

Detection of sperm mitochondrial membrane potential

Sperm were incubated with propidium iodide (IP, Molecular Probes® Inc, OR, USA) [0.25 mg/mL] and 3,3'dihexyloxacarbocyanine (DIOC6, Molecular Probes®) [10 nM] at 37°C for 30 minutes; sperm were washed by centrifuging at 300 g for 5 minutes. The pellet was resuspended in PBS, and the flow cytometer was read as previously standardised in the Reproduction Group (Mayorga-Torres et al., 2013; Mayorga-Torres et al., 2015a, 2015b).

Evaluation of sperm intracellular reactive oxygen species levels

Sperm were incubated with 2', 7' dichlorofluorescein di-acetate (DCFH-DA, Sigma-Aldrich, St. Louis, MO, USA) [1 μM] and IP [0.25 mg/mL] at 37 °C for 5 minutes, then washed three times, resuspended in PBS, and the reading was done in the flow cytometer following the previously established protocol (Mayorga-Torres et al., 2013; Mayorga-Torres et al., 2015a,b).

Determination of the integrity of the sperm membrane

Sperm were mixed with IP (final concentration 0.25 mg/mL) and Sybr 14 [1 μM] (LIVE/DEAD® Sperm Viability Kit, Molecular Probes®) at 37 °C for 30 minutes, washed with PBS, resuspended, and quantified in the flow cytometer (Mayorga-Torres et al., 2013; Mayorga-Torres et al., 2015a,b).

Analysis of sperm membrane lipoperoxidation

Sperm were incubated with 4,4-difluoro-4-bora-3a-4a-diaza-s-indacene (BODIPY C11 Molecular Probes® Inc, OR, USA) at 37°C for 30 minutes, then the sperm were washed once and resuspended in PBS before reading on the flow cytometer (Aitken et al., 2007; Mayorga-Torres et al., 2013; Mayorga-Torres et al., 2015a,b).

Detection of sperm chromatin integrity

Sperm were diluted in TNE buffer (TRIS-HCl, NaCl, EDTA-disodium, pH 7.4). Just before reading the sample in the flow cytometer, 400 μL acidic detergent solution (HCl, NaCl, Triton X-100, pH: 1.2) was added, and 30 seconds later, the acridine orange dye solution (Sigma-Aldrich, St. Louis, MO, USA, 0.006 mg/mL) was added. Finally the flow cytometer reading was performed following the previously established protocol (Aitken et al., 2007; Mayorga-Torres et al., 2013; Mayorga-Torres et al., 2015a, Mayorga-Torres et al., 2015b).

Statistical analysis

The study data were tabulated in Excel. The FREQ and CORR procedures performed statistical analyses using the SAS V.8 statistical program (SAS Institute Inc., Cary, NC, USA). Variables were analysed using descriptive statistics, Pearson's correlation was used for continuous variables. A value of $P<0.05$ was considered statistically significant.

Results

In total, eight males from four holdings in the Department of Antioquia, of different breeds and ages, underwent andrological evaluation, as shown in Table 1. In the physical evaluation, a male with hoof problems was found. In the testicular ultrasound, two males

Table 2. Variables analysed by conventional and functional analysis

Variable	n	Mean	Standard deviation	Minimum	Maximum
Scrotal circumference (cm)	8	25.69	3.22	20.0	29.0
Volume (mL)	8	0.51	0.61	0.20	2.0
Mass motility	8	3.25	1.28	1.0	5.0
Individual motility (%)	8	63.37	25.26	7.0	85.0
Concentration (million / mL)	8	1936	1456	78.0	4320.0
Vigour	8	3.6	1.27	1.0	5.0
Total abnormalities (%)	8	7.75	10.14	2.0	32.50
Necrotic cells (%)	8	39.29	27.57	13.77	96.41
Mitochondrial membrane potential-PMM (%)	8	52.94	33.62	2.31	83.52
Reactive oxygen species-ROS (%)	8	38.68	67.35	0	203.4
Membrane integrity-IM (%)	8	54.77	28.93	0	83.43
Lipoperoxidation-LPO (%)	8	7.33	9.35	0.10	27.59
DNA-DFI integrity (%)	8	12.50	2.79	8.96	16.73

Table 3. Correlation analysis between the study variables

Variable		Correlation; *P*
Scrotal circumference	Individual motility	0.846; 0.008
	ROS	-0.70; 0.050
Volume	LPO	0.821; 0.012
Individual motility	Total abnormalities	-0.88; 0.003
	Necrotic cells	-0.84; 0.008
	PMM	0.840; 0.009
	ROS	-0.91; 0.001
Total abnormalities	Necrotic cells	0.893; 0.002
	PMM	-0.88; 0.003
	ROS	0.974; <0.0001
	ME	-0.69; 0.055
Necrotic cells	PMM	-0.994, <0.0001
	ROS	0.833, 0.0102
	IM	-0.85, 0.006
PMM	IM	0.831, 0.010
ME	DFI	-0.773, 0.0245

presented lesions in the testicular parenchyma, represented in multiple hyperechoic points. The evaluation of the other organs of the reproductive system was within normal parameters.

In general, the collected semen samples presented good characteristics in the conventional and functional evaluation (Table 2). A significant positive correlation was observed for conventional and functional seminal variables: scrotal circumference and individual motility, volume and Lipoperoxidation (LPO), individual motility and Mitochondrial membrane potential (PMM), total abnormalities and Reactive oxygen species (ROS), and PMM and Membrane integrity (IM). There was a significant negative correlation for scrotal circumference and ROS, individual motility and total abnormalities, individual motility and ROS, total abnormalities and PMM, total abnormalities and IM, PMM and ROS, and LPO and DNA integrity (DFI) (Table 3).

Discussion

To the best of our knowledge, this is the first study to evaluate the conventional and functional seminal characteristics of goat semen in Colombia.

The mean values obtained for scrotal circumference (25.69 cm) are similar to the parameters reported by Revidatti et al. (2011), who reported an average of 25 and 28 cm for the Boer and Anglonubian breeds, respectively. However, there are no references for other breeds or age categories in other species (Glauber et al., 1990). Work should be done on the design of tables for each breed that relate age and optimal measurements. Scrotal circumference is important for breeder selection since it is positively correlated with individual motility and negatively with ROS production, two crucial seminal parameters.

The average semen volume of 0.51 mL and the concentration of 1936 x 10^6 sperm/mL reported in our study are lower than those reported by Almendra et al. (2005) (0.86 mL and 4791 x 10^6 sperm/mL). The mean individual motility found was 63.37%, a value that is below the report by Barkawi et al. (2006) (78.9%), Islam et al. (2006) (72.31%), and Almendra et al. (2005) (69.2%). However, the value obtained in our study could be affected by the atypical value of one of the males that presented only 7% motility. On the other hand, vigour was 4, which is higher than the 3.5 reported by Almeida et al. (2010). The percentage of abnormalities found was below those reported by Barkawi et al. (2006) (13.5%) and Islam et al. (2006) (15.9%), which shows that the results obtained for this characteristic are favourable since the percentage of morphology is an indicator strongly correlated with productive potential.

It is important to note that individual motility is one of the most predictive conventional characteristics of semen quality; it is highly correlated with functional characteristics of semen such as PMM (0.840) and ROS production (-0.91), which are predictive in fertilisation. The correlations between total abnormalities and PMM (-0.88), ROS (0.974), and membrane integrity (-0.69) behave in the same way.

The values analysed by flow cytometry showed that, on average, 52.94% of the evaluated cells present high PMM and 39.29% necrotic cells. In humans, it is estimated that a value greater than 60% in PMM is good, which suggests that the 52.94% found in this study is low. PMM is related to mitochondrial activity, which leads to sperm motility and vigour (Table 3). ROS production averaged 38.68%, and although there are no data reported for the goat species, Kadirvel et al. (2008) reported data for buffalo semen with a motility percentage greater than 70%, and a ROS of 38.4%, which may suggest

that the ROS for the evaluated males is within the ranges of the species. The results obtained for DFI show an average of 12.5% DNA fragmentation, a low value representing good quality semen, and is negatively correlated with the integrity of the membrane (-0.773), which allows for high percentages of sperm viability.

Regarding the LPO analysis, an average of 7.33% of cells were obtained with lipid oxidation; this is a functional parameter of interest since it is related to motility (Companyó et al., 2007). The MI analysis shows that, on average, 54.77% of the cells analysed have intact plasma membranes, and this parameter is correlated with sperm viability (Bonet et al., 2015). The results obtained may be undervalued since the semen of the Alpine breed presented cell death when processed in the laboratory.

In conclusion, although data regarding the semen quality of male goats are scarce, these results allow us to have references of the predictive value of the semen quality of the species in the conditions evaluated with the results found in this study. It is recommended that both conventional and functional analyses should be performed on all male goats used in artificial insemination programmes to ensure the quality of the packed semen, or that they will be left as breeder on a goat farm.

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Konvencionalna i funkcionalna procjena sjemena mužjaka mliječnih koza

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Procjena reproduktivnih indikatora u stadima koza u Kolumbiji fokusirana je na mužjake i od osnovne je važnosti za postizanje optimalnih produktivnih parametara. Cilj je ove studije bio ocijeniti kvalitetu sjemena jaraca kroz konvencionalnu i funkcionalnu analizu sjemena. Prikupljeni su uzorci sjemena osam jaraca te su procijenjeni konvencionalni (volumen, izgled, boja, pokretljivost, vitalnost i morfologija) i funkcionalni (integritet stanične membrane, potencijal mitohondrijske membrane, integritet DNK, peroksidacija lipida i proizvodnja reaktivnih spojeva kisika) parametri. Rezultati su pokazali prosječni opseg skrotuma od 25,69 cm, 0,51 mL za volumen sjemena, 1936 x 10^6 spermija/mL za koncentraciju. Prosječna pojedinačna pokretljivost bila je 63,37 %, vitalnost je bila četiri, a postotak abnormalnosti bio je 7,75 %. Pojedinačna pokretljivost povezana je s potencijalom mitohondrijske membrane (r=0,840, *P*=0,009) i proizvodnjom reaktivnih spojeva kisika (r=-0,91, *P*=0,001). Prosjek stanica s visokim potencijalom mitohondrijske membrane bio je 52,94 %, dok je bilo 39,29 % nekrotičnih stanica, prosječni indeks fragmentacije DNK bio je 12,5 %, proizvodnja reaktivnih spojeva kisika bila je 38,68 %, analiza lipoperoksidacije bila je u prosjeku 7,33 %, a integritet membrane 54,77 %. Rezultati utvrđuju parametre za sjeme antioquia koza i potvrđuje da sjeme jaraca predstavlja dobru kvalitetu i moglo bi se koristiti u programima umjetne oplodnje.

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